The impact of the CB2-63 polymorphism on the histological presentation of chronic hepatitis B

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Abstract

The impact of the cannabinoid receptor 2 (CB2) rs35761398 polymorphism on chronic hepatitis B (CHB) was evaluated in 106 consecutive biopsy-proven CHB patients naive for antiviral therapy. A histological activity index (HAI) > 8 (Ishak scoring) was more frequent in patients with CB2-63 RR than in those with CB2-63 QR or QQ (37% vs. 16.7%, $p < 0.05$). The logistic regression analysis identified CB2-63 RR ($p < 0.05$) and a fibrosis score >3 ($p < 0.005$) as independently associated with an HAI >8. The observation that the CB2-63 RR variant is an independent predictor of extensive necroinflammation opens up new prospects in the study of CHB.

Keywords: Cannabinoid receptor 2, cannabinoid system, chronic hepatitis B, HBV infection, liver disease
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Introduction

Comorbidities and several lifestyle, virus- and host-related factors have been linked to the severity of chronic hepatitis B (CHB) [1–5]. In patients with chronic hepatitis C virus infection, recent studies demonstrated an association between the severity of the liver disease and the rs35761398 polymorphism of the cannabinoid (CB) receptor 2 gene, which leads to the substitution of glutamine Gln (Q) with arginine Arg (R) at codon 63 [6,7]. In vitro studies indicated that this polymorphism may play a role in the pathogenesis of chronic hepatitis C by affecting the ability of CB receptor 2 to exert its inhibitory function, because T lymphocytes from CB2-63 RR homozygotes showed an approximately two-fold reduction in the endocannabinoid-induced inhibition of proliferation compared to cells from CB2-63 QQ homozygotes [8,9].

Materials and methods

The present investigation evaluated whether the CB2 63 variants might affect the clinical presentation and predict the outcome of CHB in 106 hepatitis B surface antigen/hepatitis B virus (HBV) DNA-positive consecutive, asymptomatic Caucasian patients naive for antiviral therapy. Patients were enrolled from July 2009 to December 2013 at the time they underwent a percutaneous liver biopsy under ultrasound guidance using a modified Menghini needle. The need for liver biopsy was established in each case by the physicians in care, and informed consent was obtained. No patient had any complication after liver biopsy. Liver specimens of at least 2 cm in length and with more than 11 portal tracts were obtained and examined by a skilled pathologist who, unaware of clinical data, graded necroinflammation and fibrosis by the Ishak scoring system [10] and liver steatosis by a partially modified Kleiner scoring system [11,12] (score 1 for fatty deposition in 1% to 10% of hepatocytes, score 2 in 11% to 31%, score 3 in 31% to 60% and score 4 in >60%). Patients with antibodies to HIV, hepatitis D virus or hepatitis c virus and those with autoimmune hepatitis were excluded. The demographics, risk factors for parenteral/sexual transmission of infections and lifestyle habits were recorded in a precoded questionnaire. The study was approved by the Ethics Committee of the AOU-Second University of Naples.

Plasma samples of all patients were tested for HBV DNA and HBV genotype as previously described [13]. Molecular screening for the *CNR2* rs35761398 polymorphism (CAA/CGG) underlying the CB2 Q63R substitution was performed

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using a TaqMan assay (Real Master Mix Probe, 5 PRIME, Germany) [6].

Continuous variables were summarized as mean and standard deviation and evaluated by an unpaired Student *t* test, and categorical variables as absolute and relative frequencies and evaluated by a χ^2 test. Nonnormally distributed variables were log-transformed. A general linear model was used for the multivariate analysis. Statistical analysis was performed using Statgraphics CENTURION XV.II (Adalta, Arezzo-Italy).

Results

The majority of the patients enrolled were infected with HBV genotype D (88.7%) and were hepatitis B e antigen–negative (95.2%). The median age was 47 years (range 21 to 69), and male subjects predominated (74.5%). Nine (8.49%) patients had a history of previous intravenous drug use and 10 (9.4%) of active alcohol abuse (more than 30g/d for female and 40g/d for male subjects over the last 6 months). The prevalences of patients showing CB2-63 QQ, QR and RR variants were 9.4%, 41.5% and 49.1%, respectively, according to the Hardy-Weinberg equilibrium (p 0.875). The demographic, biochemical, virological and histological data analysed according to the CB2-63 variants are shown in Table 1. The patients with the CB2-63 RR variant had a higher histological activity index (HAI) than those with the CB2-63 QR or QQ variant (mean 7.27 ± 3.16 vs. 5.84 ± 2.93 and 6.1 ± 1.49, respectively), differences not statistically significant probably because of the low number of QQ patients. In fact, when this analysis considered RR vs. QR+QQ subjects, the difference was significant (7.27 ± 4.08 for RR vs. 5.98 ± 4.00 for QR+QQ; p 0.0263). Moreover, moderate or severe necroinflammation (HAI >8) was more frequent in the 52 patients with CB2-63 RR than in the 54 with CB2-63 QR or QQ (37% vs. 16.7%, $p < 0.05$). The patients with the CB2-63 QQ variant showed a higher steatosis score (1.9 ± 2.2) than those with the CB2-63 RR or QR variant (0.9 ± 0.94 and 1.1 ± 0.99, respectively; $p < 0.05$), but the percentage of cases with severe steatosis (score>3) were similar in these variant subgroups, possibly suggesting that worsening in steatosis may be independent of the CB2-63 variants.

Patients with an HAI >8 showed a higher fibrosis score (mean 3.66 ± 1.24 vs. 2.44 ± 1.3, $p < 0.0001$) and a higher prevalence of patients with the CB2-63 RR variant (67.9% vs. 42.3%, $p < 0.05$) (Table 2). A multivariate logistic regression analysis including the CB2-63 variants (CB2-63 RR vs. others), fibrosis scores, age, sex, body mass index and HBV DNA showed that the CB2-63 RR variant and a fibrosis score >3 were the only independent predictors of necroinflammation >8 ($p < 0.05$ and $p < 0.005$, respectively) (Supplementary Table 1).

Discussion

The present study, the first investigating the role of the cannabinoid receptor type 2 in CHB, showed the CB2-63 RR variant to be independently associated with the more severe degrees of necroinflammation. In previous studies the CB2-63 RR variant was associated with some autoimmune pathologies such as celiac disease and childhood immune thrombocytopenic purpura

TABLE 1. Demographic, biochemical, histological and virological characteristics in HBsAg–positive patients according to the CB2-63 variants

	CB2 RR	CB2 QR	CB2 QQ	p*
Number of patients	52	44	10	—
Mean age (±SD)	46.17 ± 10.7	47.4 ± 11.5	45.2 ± 14.25	0.800
Male, n (%)	39 (75)	34 (77.27)	6 (60)	0.765
Alcohol abusers (>30 g/d), n (%)	4 (7.69)	4 (9)	2 (20)	0.811
Intravenous drug users, n (%)	5 (9.61)	3 (6.8)	1 (10)	0.878
Body mass index (mean ± SD)	25.8 ± 3.9	26.17 ± 3.6	25.9 ± 3.0	0.921
AST, IU/L (mean ± SD)	74 ± 53	78 ± 6	69 ± 32	0.946
ALT, IU/L (mean ± SD)	111 ± 89	134 ± 99	102 ± 53	0.552
Total cholesterol (mean ± SD), mg/dL	178 ± 32.6	176 ± 32.9	187 ± 26.0	0.55
Triglycerides (mean ± SD), mg/dL	93.4 ± 31.7	98.2 ± 33.3	85.1 ± 22.2	0.075
Iron (mean ± SD), ug/dL	102.2 ± 46.5	104.6 ± 34.9	106.5 ± 13.9	0.128
Ferritin (mean ± SD), ng/mL	112.1 ± 89.6	126.1 ± 115.0	136.2 ± 88.1	0.382
γGT (mean ± SD), IU/mL	42.3 ± 48.2	41.9 ± 23.16	48.7 ± 31.46	0.845
Glucose (mean ± SD), mg/dL	91.75 ± 22.56	90.0 ± 18.85	91.9 ± 8.6	0.299
HBV DNA (mean ± SD)	2,829,170 ± 12,273,183	7,769,350 ± 1,035,956	109,808 ± 33,429,955	0.698
HBV genotype, n (%)				
D	26 (90.4)	22 (93.2)	8 (90)	0.8766
Non-D	5 (9.6)	3 (6.8)	1 (10)	
HAI (mean ± SD)	7.269 ± 3.16	5.84 ± 2.93	6.1 ± 1.49	0.117
Fibrosis (mean ± SD)	2.85 ± 1.46	2.86 ± 1.41	2.69 ± 0.96	0.442
Steatosis (mean ± SD)	0.922 ± 0.94	1.051 ± 0.99	1.91 ± 2.18	0.0323

AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, γ-glutamyltransferase; HBV, hepatitis B virus; HAI, histological activity index.
*p Value still significant after correcting for multiple testing.

TABLE 2. Demographic, biochemical, histological and genetic characteristics in HBsAg–positive patients according to the HAI

	HAI<0–8	HAI >8	p*
Number of patients	78	28	
Mean age (±SD)	45.98 ± 11.85	48.28 ± 9.64	0.358
Male, n (%)	59 (75.6)	20 (71.4)	0.852
Alcohol abusers (>30 g/d), n (%)	8 (10.25)	2 (7.14)	0.91
Intravenous drug users, n (%)	9 (11.5)	0	0.13
Body mass index (mean ± SD)	26.08 ± 3.5	25.6 ± 4.26	0.559
AST, IU/L (mean ± SD)	71 ± 52	82 ± 48	0.383
ALT, IU/L (mean ± SD)	3.2 ± 3.42	2.79 ± 1.77	0.547
γGT (mean ± SD), IU/mL	45.88 ± 42.47	41.3 ± 18.76	0.583
Total cholesterol (mean ± SD), mg/dL	176.98 ± 31.8	181.42 ± 33.33	0.597
Triglycerides (mean ± SD), mg/dL	98.2 ± 32.7	90.28 ± 27.95	0.279
Iron (mean ± SD), ug/dL	108.8 ± 38.01	112.44 ± 44.45	0.703
Ferritin (mean ± SD), ng/mL	146.6 ± 109.9	148.32 ± 77.74	0.939
Glucose (mean ± SD), mg/dL	92.35 ± 19.27	90.39 ± 22.4	0.659
HBV DNA, IU/mL (mean ± SD)	5,485,060 ± 2,128,680	6,587,478 ± 1,961,599	0.782
HBV genotype, n (%)			
D	72 (92.3)	25 (89.3)	0.789
Non-D	6 (7.7)	3 (10.7)	
CB2-63 QQ, n (%)	9 (11.5)	1 (3.6)	0.38
CB2-63 QR, n (%)	36 (46)	8 (28.57)	0.16
CB2-63 RR, n (%)	33 (42.3)	19 (67.85)	0.035
Fibrosis (mean ± SD)	2.44 ± 1.3	3.66 ± 1.24	0.000
Patients with fibrosis score 5 or 6, n (%)	6 (7.69)	8 (28.6)	0.13
Steatosis (mean ± SD)	1.15 ± 1.26	0.89 ± 0.916	0.320
Patients with steatosis score 3 or 4, n (%)	9 (11.5)	2 (7.1)	0.416

HAI, histological activity index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, γ-glutamyltransferase; HBV, hepatitis B virus.
*p Value still significant after correcting for multiple testing.

[14–16]. Accordingly, the mechanism of liver cell necrosis in acute and chronic hepatitis B resembles that of autoimmune hepatitis, because HBV is not directly cytopathic and liver cell necrosis is due to a cell-mediated immune reaction in which the presensitized cytotoxic T-cells bind to the HBV core antigen and to a human leukocyte class II antigen on the surface of HBV-infected hepatocytes and introduce necrotizing cytokines to these cells [17]. In addition, endocannabinoids and their receptors play an important role in other chronic liver diseases, because they have been found to be involved in the pathogenesis of liver fibrosis in alcohol-related hepatitis, in obesity-related liver steatosis [18–20] and in chronic hepatitis C [6,7]. In line with these studies, our data identified an independent association between the CB2-63 RR variant and more extensive liver cell necroinflammation in CHB. We concede that the sample size of this study does not allow definitive conclusions to be drawn and that the data presented need confirmation. It does, however, open up new promising prospects in the study of CHB.

Transparency declaration

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2015.02.021.

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